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REMARKS

I. Status of the claims and support for amendments

Claims 31 and 41 are amended.

Claims 38–40 are withdrawn.

Claims 31 and 37–41 are currently pending.

II. Amendment to the Specification

The Action alleges that the Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. §120 (claiming the benefits of an earlier application). Applicant notes that as part of the Preliminary Amendment, filed May 9, 2001 (receipt of which was acknowledged by the USPTO and listed at the public Patent Application Information Retrieval system) priority was claimed as required. Consequently, the amendment to the Specification is made merely to update the Application with the current status of the parent application.

III. Election/Restriction

Applicant acknowledges, with thanks, the fact that claims 38–40 are currently withdrawn, but will be rejoined once generic claim 41 is found to be patentable.

IV. Rejection under 35 U.S.C. §112

A. New Matter Rejection

Claims 31, 37, and 41 are rejected under 35 U.S.C. §112 as allegedly containing "new matter" for their use of the phrase "*specifically hybridizes*". Applicant respectfully traverses.

As currently amended, claim 41 now recites, in pertinent part "specifically hybridizes under stringent condition". For the reasons set out below, Applicant believes that this amendment overcomes the "new matter" objection.

As described in the *MPEP* a new matter rejection of a claim is proper when "*subject matter not disclosed in the original application is...added and ...claim[ed]*". *MPEP §706.03(o)*. However, it is Applicant's position that the claimed subject matter was disclosed in the original application. It has been held that: "[a]dequate description under the first paragraph of 35 U.S.C. § 112 does not require literal support for the claimed invention....Rather, it is sufficient if the originally-filed disclosure would have conveyed to one having ordinary skill in the art that an appellant had possession of the concept of what is claimed." *Ex parte Parks*, 30 USPQ2d 1234, 1236-37 (B.P.A.I. 1993) (emphasis added). Moreover, the Circuit Court of Patent Appeals reversed a new matter rejection based on appellant's change of the claim terminology "*containing* a medicant" to "*carrying* a medicant". The court reasoned: "[t]he question, as we view it, is not whether "carrying" was a word *used* in the specification as filed but whether there is support in the specification for employment of the term in a claim; is the concept of carrying present in the original disclosure? We think it is." *In re Anderson*, 176 USPQ 331, 336 (CCPA 1973) (emphasis original). Thus it is evident that literal or *ipsis verbis* support for claim language is not required. All that is required is that the subject matter be disclosed in the application as originally filed. Applicant believes that this standard is met.

Firstly, it is Applicant's position that emphasis is improperly placed on the phrase "specifically hybridizes". Clearly there is support, in many locations in the specification, for the use of the term "hybridizes" To Applicant's view the issue is whether the term "*specifically*" is disclosed, either explicitly or implicitly.

When viewed as a whole (particularly in view of what was common knowledge among those skilled in the art, when the application was filed), there is unmistakable support for the use of the term "specifically hybridizes". The term "specifically hybridizes" was in common use

prior to the priority date of the instant application. The term was well understood by those skilled in the art to mean that hybridization occurred under stringent conditions and was specific or selective for those sequences that were very closely related or identical to the probe sequence. For example, see Hahn *et al.*, *Nature*, 300:184–186 (1987), which is cited in the Information Disclosure Statement and is referred to at page 2 of the specification (*see* page 2, lines 27–29). The abstract for the Hahn *et al.* reference (a copy of which is enclosed for the Examiner's convenience) refers to "*specific DNA probes*". Moreover, use of the terms "*specific hybridization*" and "*hybridizes specifically*" was commonplace, prior to the filing of the priority document for the instant application. A few examples include: (1) Kahn and Wright, *J. Virol. Methods* (1987), 15:121–130; (2) Bodner *et al.*, *PNAS USA*, (1985), 82:3548–51; (3) Snead *et al. J. Biol. Chem.* (1981), 256:11911–16; (4) Scarpulla *et al. J. Biol. Chem.* (1981), 256:6480–86; and Law *et al.*, *J. of Virology*, (1979), 32:199–207 (for the Examiner's convenience copies of the pertinent parts of each of these articles, showing the use of these terms).

In summary, the use of the term "specific" with respect to nucleic acid probes is explicitly disclosed (page 2 of the specification) and the term "specifically hybridizes" was well known and widely used by those of skill in the art at the time the application was filed.

Additionally, the specification provides further implicit support for the use of this term. At page 2 the specification describes specific probes as "hybridiz[ing] preferentially" to the target DNA. The meaning of this language is further elucidated by the disclosure at page 5, lines 5–8, which recite, in pertinent part, "the unique characteristics of HIV-3 can best be appreciated by comparison with the same type of characteristics relating to other human immunodeficiency viruses, HIV-1 and HIV-2". An example of this type of comparison is illustrated by the results shown in Figure 14. Figure 14 (*see* description on page 9 of the specification) shows that under

"stringent conditions" there is no cross-hybridization between HIV-1, HIV-2, and HIV-3; that is, the hybridization is "specific". In contrast, under non-stringent conditions, cross-hybridization, or non-specific, hybridization occurs. Moreover, the meaning of the terms "stringent" and "non-stringent" (as is discussed fully in section IV. C., below) are described at pages 17 and 37 respectively.

In view of the foregoing explanation, Applicant asserts that though the term "specifically hybridizes" may not appear *ipsis verbis* there is, nevertheless, explicit support for this term. Furthermore, even if one is not convinced that there is explicit support for the use of this term, the presence of implicit support for the use of the term is unequivocal. Accordingly, Applicant asserts that the standards set out in *Ex parte Parks* and *In re Anderson* as sufficient to obviate a "new matter" rejection are fully met with by the instant Application, as pertaining to the use of the term "specifically hybridizes". Further, the addition of the phrase "under stringent conditions" to claim 41 immediately subsequent to the term "specifically hybridizes" renders the terms meaning clear, from context alone, to one skilled in the art. Consequently, Applicant believes that this rejection for new matter has been overcome and may now properly be withdrawn.

B. Written Description Rejection

Claims 31 and 41 are rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter that is not described in the specification in such a way as to reasonably convey to one of skill in the art that the inventors, at the time the application was filed were in possession of the claimed invention. Specifically, the rejection alleges that there is not sufficient support to "*encompass probes that specifically hybridize to genomic RNA of the HIV-3 retrovirus deposited at the European Collection of Animal Cell Cultures (ECACC) under No. V88060301).*

None of these sequences meet the written description provision of 35 USC 112, first paragraph. The specification provides insufficient written description to support the genus encompassed by the claim." Applicant respectfully traverses.

Applicant wishes to begin by drawing the Examiner's attention to a decision by the Court of Appeals for the Federal Circuit in 2002 that directly addresses this written description issue, namely, *Enzo Biochem, Inc. v. Gen-Probe Inc.*, 296 F.3d 1316 (Fed. Cir. 2002) (for the Examiner's convenience, a copy of this case in enclosed herewith). At issue in *Enzo* was whether deposits of biological material in a public depository provided the necessary written description for claims drawn to nucleic acid probes that selectively hybridize to the genetic material of the bacteria that cause gonorrhea. *Id.*, at 1322. In finding for Enzo Biochem., Inc. the court stated:

> [w]hile deposit in a public depository most often has pertained to satisfaction of the enablement requirement, we have concluded that reference in the specification to a deposit may also satisfy the written description requirement with respect to a claimed material. . . .We therefore agree with Enzo that reference in the specification to deposits of nucleotide sequences describe those sequences sufficiently to the public for purposes of meeting the written description requirement."

Id., at 1326 (emphasis added). Thus *Enzo* directly addresses the instant issue. Since *Enzo* was decided subsequent to all of the cases cited in the Office Action it supercedes their holdings and provides precedence for the matter at issue.

In view of the decision in *Enzo* Applicant asserts that the deposited material provides the written description required by 35 U.S.C. §112, first paragraph. As in *Enzo*, the presently rejected claims refer to nucleic acid available through a publicly available biological deposit. The claims refer to a DNA probe that hybridizes with the genomic RNA of the HIV-3 retrovirus that has been deposited. The fact that the retrovirus was deposited would convince one of

ordinary skill in the art that the inventors were in possession of the invention at the time the application was filed.

The rejection also asserts that "while SEQ ID NO:1 is disclosed, the specification is silent as to whether said sequence will meet the functional limitations of the rejected claims." Applicant respectfully disagrees. As part of the Amendment, mailed December 20, 2001, in response to the requirement for a corrected Sequence Listing, the specification was amended so that page 52, line 2, recites "HIV-3LTR (SEQ ID NO:1)". Further, page 51 states that SEQ ID NO:1 corresponds to sequence from clone iso 70-11. Page 19, lines 23–25 disclose that iso 70-11 is a clone containing a large insert from HIV-3. The isolation of clone iso 70–11 is described at pages 17–19 of the specification, where it states, *inter alia*, that it was isolated from the HIV-3 strain ANT 70. Finally, at page 5, line 16, the specification discloses that the ANT 70 strain was the one deposited as ECACC V88060301. Accordingly, SEQ ID NO:1 clearly falls within the limitations of claims 31 and 41.

Thus, given that the court held in *Enzo* that a deposit of biological material provides the written description required to support a claim and that the genetic material on deposit is the clear origin of the sequence provided in SEQ ID NO:1, Applicant contends that the rejections of claims 31 and 41, for lack of written description, has been overcome and may now properly be withdrawn.

C. Enablement Rejection

Claims 31, 37, and 41 are rejected under 35 U.S.C. §112, first paragraph as allegedly not being enabled by the specification. The rejection alleges that:

> claims 31 and 41 encompass polynucleotides (DNA probes) comprising non-disclosed nucleic acid sequences that **specifically hybridize** to the genomic RNA of HIV-3 retrovirus [and] the specification fails to specifically define what parameters constitute "stringent conditions". Therefore, said term is not limiting.

> As disclosed above, the specification dose not teach how to make any polynucleotides that specifically hybridize to the genomic RNA of the HIV-3 retrovirus... . Clearly since the specification has not taught how to make/use said polynucleotides, the specification has not enabled the instant claims that require DNA probes that specifically hybridize to the genomic RNA of the HIV-3 retrovirus... .When given the broadest reasonable interpretation, the claims are clearly intended to encompass a variety of species including full-length cDNAs, genes and protein coding regions. Moreover, the use of the term "comprising" (claim 37 and "contains" (claim 31 reads on intact genomic material comprising enhancers, promoters, introns, and splice sites, etc. No reading frames are identified in any sequence such that one of skill in the art would be able to determine where such features could be within the sequence. Clearly, it would be expected that a substantial number of the hybridizing or complementary polynucleotides encompassed by the claims would not share either structural or functional properties with the polynucleotides that encode SEQ ID NO:1 or its complement. The specification fails to provide an enabling disclosure for how one would make such polynucleotides. The specification provides insufficient guidance with regard to these issues and provides no working examples that would provide guidance to one skilled in the art on how to make/use the broadly claimed genus. For the above reasons, undue experimentation would be required to practice the claimed invention.

Applicant respectfully traverses.

With respect to the current claims recitation of the phrase "specifically hybridizes under stringent conditions", Applicant notes that the use of the term "specifically hybridizes" is fully discussed in part IV. A. of this response and the arguments there apply, with equal effect, here.

The rejection also alleges that the term "stringent conditions" is not defined in the specification. Applicant respectfully disagrees. It is well understood by those skilled in the art that the "stringency" of a nucleic acid hybridization refers to the conditions under which the hybridization and washing of the hybridization are performed. Specifically, it is determined in terms of salt concentrations (with higher salt concentrations being less stringent), the presence or absence of denaturants or hydrogen bond disrupting agents, such as formamide (with high concentrations providing a more stringent condition), and temperature (higher temperatures being more stringent). The present application clearly defines what is meant by stringent and non-stringent conditions. For example, definitions of stringent conditions are provided at page

17, lines 16–23, page 37, lines 24–31, and page 40, lines 10–21. Similarly, non-stringent conditions are defined at page 37, lines 33 through page 38, line 2. Thus, the meaning of stringent and non-stringent is clearly defined in the specification and is, therefore, limiting.

Contrary to the allegation in the rejection, the specification unambiguously teaches how to make DNA probes encompassed by the claims. The preparation of SEQ ID NO:1 provides just such an example. The preparation of SEQ ID NO:1, derived from iso 70-11, is set out in detail beginning at page 16 of the specification. It would have been well within the abilities of one of skill in the art at the time the Application was filed to follow the methods set out beginning at page 16 of the specification and using a sample of the biological deposit V88060301 to prepare other probes, without undue experimentation. In fact, as the Examiner has aptly stated, the "claims are clearly intended to encompass [just such] a variety of such species . . .." Further, as the probes are intended to detect the presence of intact HIV-3 retrovirus in a sample, there is no need to limit the probe to some particular reading frame, exon, or protein. The probes are meant to detect the RNA from whole virus that, by definition, comprise the entirety of the HIV-3 genome. Consequently, it is entirely proper and desirable for the claims to read on probes to the intact genomic material.

Accordingly, the unassailable conclusion that must flow from the foregoing is that the specification clearly provides sufficient guidance allowing those of skill in the art to prepare probes (including one comprising the sequence of SEQ ID NO:1) from the biological deposit ECACC No. V88060301, without any undue experimentation. Therefore, Applicant asserts that the rejection under 35 U.S.C. §112, first paragraph, for lack of enablement has been overcome and may now properly be withdrawn.

D. Indefiniteness Rejection

Claims 31, 37, and 41 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite.

Claim 31 is alleged to be indefinite for its use of the phrase "at least 360 contiguous sequences corresponding to the genomic RNA of HIV-3". In response Applicant has amended claim 31 to recite "360 contiguous nucleotides". Applicant believes that this amendment fully addresses and overcomes the rejection. Accordingly, Applicant respectfully requests that this rejection be withdrawn.

Claim 41 is rejected as allegedly being vague and indefinite for its use of the phrase "stringent conditions". The rejection alleges that "it is unclear what parameters are encompassed by said phrase as the specification fails to provide a definition." Applicant respectfully traverses.

As detailed in section IV. C., *supra*, the specification fully defines both the terms "stringent hybridization conditions" and "non-stringent hybridization conditions." The specification discloses that the parameters encompassed include among, others, salt concentration and temperature. Thus, it is Applicant's position that the term "stringent hybridization conditions" is fully and definitively defined. Consequently, Applicant respectfully requests that this rejection be withdrawn.

Claim 41 is rejected as allegedly being indefinite for its use of the term "specifically hybridizes". The rejection alleges that it is unclear what is meant by the term and queries as to whether the word "specificity" refers to the deposited material or HIV-3 generally. Applicant respectfully traverses.

As detailed in section IV. A., *supra*, the term "specifically hybridizes" is well known and widely used by those of skill in the art. Accordingly, it is Applicant's position that this term is

clear and definite. Moreover, as described above and clarified by amendment of claim 41 to recite "specifically hybridizes under stringent hybridization conditions". This means that if mixed and hybridized under stringent hybridization conditions the probe used would specifically hybridize with the RNA of the deposited HIV-3 virus. That is, under those stringent hybridization conditions it would "recognize" or bind to HIV-3, but not HIV-1 or HIV-2, for example. Nevertheless, this places no limitation on the type or strain of HIV-3 retrovirus (or its RNA) in the "biological liquid or tissue" set out in claim 41. That is, it does not limit the strain of HIV-3 that might be detected by the claimed process.

V. Rejection under 35 U.S.C. §102

Claims 31 and 41 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Montagnier *et al.* (WO 86/02383). The rejection alleges that Montagnier *et al.* disclose methods for the use of DNA hybridization probes for the detection of LAV (HIV) in tissues and fluids. It is deemed in the absence of evidence to the contrary, that one of the probes encompassed by the Montagnier *et al.* disclosure will be effective in the detection of HIV-3 or its RNA since the hybridization conditions and the limitation "specifically" have not been defined. Applicant respectfully traverses.

As currently amended, claims 31 and 41 are limited to processes that require probes that "specifically hybridize under stringent conditions" with the deposited HIV-3 RNA. As is shown by the data presented in Figure 14. This results in a probe that recognizes HIV-3 but not HIV-1 or HIV-2. Further, probes for HIV-1 or HIV-2 do not "recognize" HIV-3 under these conditions. Accordingly, Montagnier *et al.* cannot anticipate the present claims because it does not teach or describe probes capable of "specifically hybridizing [to HIV-3] under stringent conditions . . .." Finally, it is Applicant's position that the limitation "specifically" is fully defined, *see* section

IV, *supra*. In view of the foregoing, Applicant believes that the rejection of claims 31 and 41 as being anticipated under 35 U.S.C. §102(b) by Montagnier *et al.* has been overcome and may now properly be withdrawn.

VI. Conclusion

In view of the foregoing Amendments and Remarks, Applicant believes that all rejections and objections have been overcome and that the instant application is now in condition for immediate allowance. Consequently, Applicant respectfully requests favorable reconsideration of the application and issuance of a Notice of Allowance therefore.

The Examiner is invited to contact the undersigned attorney at (713) 787-1589 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

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Date: July 20, 2004

1: Nature. 1987 Nov 12-18;330(6144):184-6.

nature

Relation of HTLV-4 to simian and human immunodeficiency-associated viruses.

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Human immunodeficiency virus type 1 (HIV-1) is the aetiologic agent of AIDS (acquired immune deficiency syndrome) in most countries and probably originated in Central Africa like the AIDS epidemic itself. Evidenc for a second major group of human immunodeficiency-associated retroviruse came from a report that West African human populations like wild-caught African green monkeys had serum antibodies that reacted more strongly with a simian immunodeficiency virus (STLV-3Mac) (ref.6) than with HIV-1. Novel T-lymphotropic retroviruses were reported to have been isolated from healthy Senegalese West Africans (HTLV-4) (ref. 4) and from African green monkeys (STLV-3AGM) (ref. 7), and a different retrovirus (HIV-2) was identified in other West African AIDS patients. Genomic analysis of HIV-2 clearly distinguished it from STLV-3 (ref. 9), but restriction enzyme site-mapping of three different HTLV-4 isolates and six different STLV-3AGM isolates showed them to be essentially indistinguishable. In this report we clone, restriction map, and partially sequence three isolates of HTLV-4 (PK82, PK289, PK190) (ref. 4). We find that these viruses differ in nucleotid sequence from each other and from three isolates of STLV-3AGM (K78, K6W, K1) (ref. 7) by 1% or less. We also report the isolation of a T-lymphotropic retrovirus from the peripheral blood of a healthy Senegalese woman which hybridizes preferentially to HIV-2 specific DNA probes. We conclude that HTLV-4 (ref. 4) and STLV-3AGM (ref. 7) are not independent virus isolates and that HIV-2 is present in Senegal as it is in other West African countries.

PMID: 2823148 [PubMed - indexed for MEDLINE]

Jul 8 2004 06:47:

1: J Virol Methods. 1987 Feb;15(2):121-30.

Detection of flavivirus RNA in infected cells using photobiotin-labelled hybridization probes.

Khan AM, Wright PJ.

Ten plasmids containing viral cDNA inserts of portions of the dengue virus type 2 (DEN-2) or Kunjin virus (KUN) genomes were biotinylated using photobiotin acetate and used as probes for the detection of flavivirus RNA in infected Vero cells. The viral cDNA inserts ranged in length from 0.19 to 2.7 kilobase pairs, and represented segments of the flavivirus genome coding for structural and nonstructural proteins. In spot hybridization assays (hybridization at 60 degrees C) with RNA extracted from cells infected with one of fourteen different flaviviruses or Semliki Forest virus, all DEN-2 and KUN probes hybridized specifically with RNA from cells infected with DEN 2 or KUN, respectively. At the reduced stringency of lower temperatures, specific hybridization to homologous viral RNA was still a feature of the probes, and only limited cross-hybridization to the RNA of some other flavivirus species was detected.

PMID: 3031110 [PubMed - indexed for MEDLINE]

1: Proc Natl Acad Sci U S A. 1985 Jun;82(11):3548-51.

Coding sequences for vasoactive intestinal peptide and PHM-27 peptide are located on two adjacent exons in the human genome

Bodner M, Fridkin M, Gozes I.

The human precursor gene for vasoactive intestinal peptide (VIP) and PHM-27, a peptide that has an NH2-terminal histidine and COOH-terminal methionine amide and is closely related in sequence and activity to VIP, was detected with synthetic oligodeoxynucleotide probes. These specific hybridization segments were constructed according to the neuroblastoma VII cDNA sequence and contained up to 39 bases. The gene structure was partly deduced by hybridization to synthetic oligodeoxynucleotide probes and partl by direct chemical nucleotide sequencing. Four exons were discovered thus far; among them are two short exons separated by a 0.75-kilobase DNA stretch, one encoding PHM-27 and the second encoding VIP (exons 1 and 2) Each of these two exons encodes both the hormone amino acid residues as well as the post-translational processing signal sequences. The 3' splice sites of the two exons contain an identical stretch of nine nucleotides. At the cDN level, the 3' splice sites contain the same stretch of six nucleotides, which are identically spliced. The occurrence of VIP and PHM-27 coding sequences or two separate exons of the human genome and the homology of their 3' splice site may allow alternative RNA processing as discussed below.

PMID: 2987932 [PubMed - indexed for MEDLINE]

1: J Biol Chem. 1981 Nov 25;256(22):11911-6.

Mosaic structure and mRNA precursors of uteroglobin, a hormone-regulated mammalian gene.

Snead R, Day L, Chandra T, Mace M Jr, Bullock DW, Woo SL.

The synthesis of uteroglobin in the rabbit uterus is induced by progesterone and is repressed by estrogen which has an over-riding effect over the inducer The dual hormonal control system offers an excellent model for studying hormonal regulation of mammalian gene expression. Using a full-length uteroglobin cDNA clone as a specific hybridization probe, recombinant lambda phages containing the entire chromosomal uteroglobin gene have been isolated from a rabbit genomic DNA library. Electronmicroscopic analysis of hybrid molecules formed between the chromosomal uteroglobin gene and uteroglobin mRNA indicated the presence of 2 intervening sequences within this gene. The mosaic structure of the uteroglobin gene has been substantiated by detailed restriction mapping and Southern hybridization. The gene is 3.0 kilobases in length to code for a mature mRNA of 465 nucleotides. Northern hybridization of poly(A)-containing RNA from 4-day-pregnant rabbit uterus with the full-length cDNA clone revealed the presence of uteroglobin mRNA precursors. The size of the largest precursor RNA species detected by the cDNA clone is the same as the entire chromosomal uteroglobin gene. The fidelity of the precursor RNAs was established by their ability to hybridize with specific intervening sequence probes. Thus the entire uteroglobin gene is expressed into primary RNA transcripts, which are subsequently processed into mature mRNA molecules by splicing.

PMID: 6271764 [PubMed - indexed for MEDLINE]

☐ 1: J Biol Chem. 1981 Jun 25;256(12):6480-6.

Isolation and structure of a rat cytochrome c gene.

Scarpulla RC, Agne KM, Wu R.

We screened a Charon 4A-rat genomic library using the cloned iso-1 cytochrome c gene from Saccharomyces cerevisiae as a specific hybridizatio probe. Eight different recombinant phages homologous to a coding region subfragment of the yeast gene were isolated. Nucleotide sequence analysis of a 0.96-kilobase portion of one of these established the existence of a gene coding for a cytochrome c identical in amino acid sequence with that of mouse. The rat polypeptide chain sequence had not previously been determined. In contrast to the yeast iso-1 and iso-2 cytochrome c genes, neither of which have introns, the rat gene contains a single 105-base pair intervening sequence interrupting glycine codon 56. The overall nucleotide sequence homology between cytochrome c genes of yeast and rat is about 62%, with areas of greater homology coinciding with four regions of functionally constrained amino acid sequences. Two of these regions displayed 85-90% DNA sequence homology, including the longest consecutive homologous stretch of 14 nucleotides, corresponding to amino acids 47-52 of the rat protein. Somewhat less homology was observed in the DNA-specifying amino acids 70-80, which are invariant residues in most known cytochrome c molecules. Thermal dissociation of the yeast probe fror the homologous rat DNA was at about 58 degrees C in 0.39 M Na+. These results establish that cytochrome c genes may be isolated by interspecies hybridization between widely divergent organisms.

PMID: 6263917 [PubMed - indexed for MEDLINE]

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Conserved Polynucleotide Sequences Among the Genomes of Papillomaviruses

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The DNAs of different members of the *Papillomavirus* genus of papovaviruses were analyzed for nucleotide sequence homology. Under standard hybridization conditions (T_m − 28°C), no homology was detectable among the genomes of human papillomavirus type 1 (HPV-1), bovine papillomavirus type 2 (BPV-2), or cottontail rabbit (Shope) papillomavirus (CRPV). However, under less stringent conditions (i.e., T_m − 43°C), stable hybrids were formed between radiolabeled DNAs of CRPV, BPV-1, or BPV-2 and the *Hin*dIII-*Hpa*I A, B, and C fragments of HPV-1. Under these same conditions, radiolabeled CRPV and HPV-1 DNAs formed stable hybrids with the *Hin*cII B and C fragments of BPV-2 DNA. These results indicate that there are regions of homology with as much as 70% base match among all of these papillomavirus genomes. Furthermore, unlabeled HPV-1 DNA competitively inhibited the specific hybridization of radiolabeled CRPV DNA to BPV-2 DNA fragments, indicating that the homologous DNA segments are common among these remotely related papillomavirus genomes. These conserved sequences are specific for the *Papillomavirus* genus of papovaviruses as evidenced by the lack of hybridization between HPV-1 DNA and either simian virus 40 or human papovavirus BK DNA under identical conditions. These results indicate a close evolutionary relationship among the papillomaviruses and further establish the papillomaviruses and polyoma viruses as distinct genera.

The Papovaviridae family consists of the papillomaviruses (genus A) and the polyoma viruses (genus B) (19). The papillomaviruses are distinguished by the larger size of the icosahedral capsid virions (55 nm versus 40 nm) and the larger size of the supercoiled, double-stranded DNA genome (5.0×10^6 daltons versus 3.3×10^6 daltons) (3, 8). Members of the *Papillomavirus* genus produce cutaneous papillomatosis and other epithelial proliferations in their natural hosts, including humans, cattle, rabbits, dogs, sheep, chaffinches, and horses, among others. Studies on the molecular biology and genetics of individual members of this genus have been limited, primarily as a consequence of the lack of a cell culture system suitable for propagating these viruses (1).

Recent studies have demonstrated a remarkable plurality of human papillomaviruses. To date, at least five discrete classes of papillomaviruses have been isolated from a variety of proliferative epithelial lesions of humans. Human papillomavirus types 1 and 4 (HPV-1 and HPV-4) are preferentially associated with deep plantar warts (2, 6, 7, 10). Human papillomavirus type 2 (HPV-2) is preferentially associated with common warts (2, 23, 24). Two other papillomaviruses (HPV-3 and HPV-5) have been isolated from the benign cutaneous proliferations of patients with epidermodysplasia verruciformis (2, 26), and, interestingly, the lesions containing HPV-5 appear to have the potential to undergo malignant transformation (25). Each of these human papillomaviruses is immunologically distinct and can be differentiated by nucleic acid hybridization and restriction endonuclease analysis of their genomes (26). Under stringent hybridization conditions little if any DNA sequence homology could be detected among any of these five classes of human papillomaviruses (24, 26). Also, under nonstringent conditions (37°C and 30% formamide) no stable heteroduplexes could be detected between the genomes of HPV-1 and HPV-2, emphasizing the distinctness of these two viruses (24).

Other animal species also contain more than one papillomavirus. In cattle, two distinct papillomaviruses were purified from bovine fibropapillomas which showed 45 to 58% nucleic acid homology when assayed under stringent conditions (14). Under similar stringent conditions no homology was detectable between the genomes of either bovine papillomavirus type 1 or type 2 (BPV-1 or BPV-2) and HPV-1 (14). The cotton-

tail rabbit (Shope) papilloma virus (CRPV) produces papillomas in cottontail rabbits (*Sylvilagus floridanus*) and is associated with squamous cell carcinoma of the skin in domestic rabbits (*Oryctolagus cuniculus*) (29, 32). A distinct papillomavirus of domestic rabbits is associated with oral papillomatosis (27). In addition, no homology is detectable between the CRPV and human papillomavirus DNAs under stringent conditions (3).

One of the difficulties with the studies to date examining the nucleotide sequence relatedness among the papillomaviruses is the stringency under which the experiments were performed. In general, homology between two papillomavirus genomes has been examined at 20 to 25°C below the melting temperature (T_m) of the genomic DNAs. Although these conditions are optimal for the kinetics of DNA-DNA reassociation (35), they are limiting when one wishes to examine sequence homology among different but similar DNAs; under these conditions only segments of DNA with at least five of six bases matched will be thermally stable (12). Homologous segments of the genome with greater base mismatch will not be detected at $T_m - 25$°C.

In this study we examined the sequence homology of several remote papillomaviruses under conditions of varying stringency. We found that at the level of 25% base mismatch homologous segments could be detected among the genomes of papillomaviruses of different species.

MATERIALS AND METHODS

Virus purification. HPV-1 was purified from a sample of plantar wart (0.12 g) kindly supplied to us by B. Jenson (National Institutes of Health). The wart was ground with sterile sand in phosphate-buffered saline, pH 7.4. The extract was centrifuged to remove sand and cell debris. The viral particles in the supernatant were purified twice by equilibrium centrifugation in a CsCl gradient (1.34 g/cm^3) followed by sedimentation once in a neutral sucrose gradient (21 to 61%). The yield of viral particles in such a plantar wart sample was approximately 2%. BPV-1 and BPV-2 were prepared as previously described (14).

Viral DNA. HPV-1 DNA was isolated from purified virions. Viral particles were disrupted by incubation with 1% Sarkosyl and DNA was extracted with phenol. The identity and homogeneity of the DNA preparation were ascertained by its mobility and its cleavage pattern with restriction endonucleases on agarose gel electrophoresis.

Purified BPV-1 and BPV-2 DNAs were prepared as described previously (15), and CRPV DNA was the generous gift of D. Lowy (National Institutes of Health).

Unlabeled DNAs of simian virus 40 (SV40) and of the MM isolate of human papovavirus BK [BK(MM)] were isolated directly from infected African green monkey kidney cells and human embryonic kidney cells, respectively, by differential salt precipitation according to the procedure of Hirt (11). The supercoiled DNA genomes were purified by isopycnic equilibrium centrifugation in a CsCl-ethidium bromide gradient (1.56 g/cm^3) followed by sedimentation through a neutral sucrose gradient (5 to 30%).

The DNAs of human and bovine papillomaviruses, CRPV, SV40, and BK(MM) were radiolabeled to high specific activity by the enzymatic reaction of *Escherichia coli* polymerase I and DNase I in the presence of [α-^{32}P]ATP and [α-^{32}P]GTP, using the nick translation procedure described by Rigby et al. (28). The specific activities of radiolabeled DNAs ranged from 50×10^6 to 100×10^6 cpm/μg.

Restriction and electrophoresis of viral DNA. Restriction endonucleases *Bam*HI, *Hin*cII, *Hin*dIII, *Hpa*I, *Eco*RI, and *Bgl*I were purchased from New England Biolabs, Lowell, Mass., or from Bethesda Research Laboratories, Rockville, Md. Each viral DNA (1 μg) was digested with 2 U of restriction endonucleases in 100 μl of the appropriate buffer at 37°C for 1 h. Sequential cleavage of viral DNAs was achieved as previously described (16).

The total volume of completely cleaved unlabeled viral DNA was loaded in a 10 cm by 0.9 cm slot in a 1.6% agarose vertical slab gel (E.C. Apparatus). The cleaved DNA fragments were separated by electrophoresis at 150 V for 3 h. These fragments were denatured in situ and were subsequently transferred and immobilized onto a nitrocellulose filter as described by Southern (33).

DNA-DNA hybridization. Strips 5 mm wide cut from the unlabeled DNA blots were preincubated at 60°C for 4 to 10 h in Denhardt's solution (5) before hybridization. Radiolabeled viral DNA probes were annealed to the unlabeled DNA immobilized on the filters at 35°C in 1 M NaCl, 0.01 M *N*-tris-(hydroxymethyl)methyl-2-aminomethane-sulfonic acid (pH 7.4), 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, and the indicated formamide concentration for 24 h as previously described (12). In each experiment there was approximately a 10-fold excess of unlabeled DNA on the blot to the probe, and the hybridization was continued to plateau levels as previously described (12). The nitrocellulose filter strips were washed extensively with 4 × SSC (0.15 M NaCl plus 0.015 M sodium citrate) at temperatures equivalent to their respective hybridization conditions and then air-dried. The blots were subsequently exposed to RP-Royal Xomat X-ray film (Kodak) for autoradiograms.

RESULTS

Detection and mapping of homology between the HPV-1 and BPV-2 genomes. To detect and map any homology between the genomes of HPV-1 and BPV-2, we annealed ^{32}P-labeled BPV-2 DNA to HPV-1 *Hin*dIII/*Hpa*I DNA fragments under a range of conditions. This combination of enzymes cleaves the HPV-1 genome into five fragments with the following map coordinates: A, 0.285 to 0.62 map units; B, 0.85 to 0.125 map units; C, 0.67 to 0.85 map units;

D, 0.125 to 0.285 map units; and E, 0.62 to 0.67 map units (6). At 35°C (T_m − 28°C) in 50% formamide, stringent hybridization conditions equivalent to those used by Lancaster and Olson (14), no stable hybrids could be detected between the radiolabeled BPV-2 DNA probe and unlabeled immobilized HPV-1 DNA fragments (Fig. 1). At the same temperature, however, stable hybrids between the radiolabeled BPV-2 DNA probe and the HPV-1 DNA fragments could be detected at lower formamide concentrations, indicating that there are regions of homology between these two DNAs with significant base mismatch. In 40% formamide (an effective temperature of T_m − 36°C for HPV-1 DNA) ^{32}P-labeled BPV-2 DNA formed a stable hybrid with the HPV-1 fragment A, indicating a region of homology within that fragment with a maximum of 25% base mismatch. Under less stringent conditions (30% formamide, which is an effective temperature of T_m − 43°C for HPV-1 DNA), additional stable hybrids formed between the radiolabeled BPV-2 probe and HPV-1 fragments B and C, indicating regions of homology with a maximum of 30% base mismatch within those two fragments. Greater hybridization occurred at the lower formamide concentrations with HPV-1 fragment C than with HPV-1 fragment B, which may indicate that the homologous regions within the HPV-1 C fragment are more extensive than within the HPV-1 B fragment. Even in 20% formamide (effective temperature of T_m − 48°C for HPV-1 DNA), however, no homology was detected between the BPV-2 probe and HPV-1 fragments D and E (Fig. 1). The locations of the five HPV-1 *HpaI/HindIII* fragments are indicated on the physical map of the genome shown in Fig. 2 (6); the noncontiguous fragments containing the regions homologous to the BPV-2 genome are indicated with bold lines.

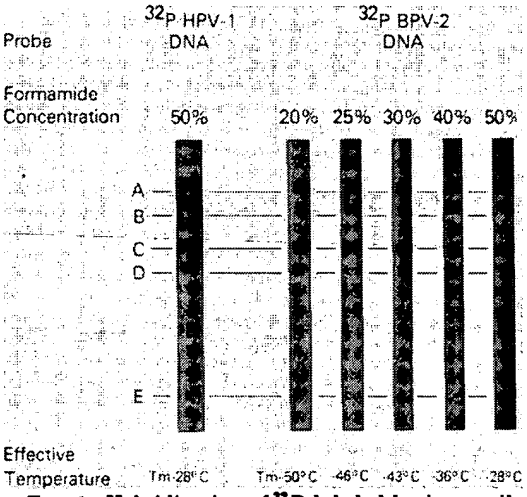

FIG. 1. *Hybridization of ^{32}P-labeled bovine papillomavirus type 2 DNA to fragments of the human papillomavirus type 1 (HPV-1) genome. Nitrocellulose filter strips (5 mm wide) containing unlabeled HindIII/HpaI fragments of the HPV-1 genome (0.05 μg per strip) were incubated for 18 h in 1 ml of a reaction mixture containing 500,000 cpm of in vitro ^{32}P-labeled and denatured HPV-1 DNA or BPV-2 DNA (specific activities were 7.0 × 10^7 cpm/μg and 9.0 × 10^7 cpm/μg, respectively) at 35°C at the indicated formamide concentrations. The strips were washed at the temperature equivalent to the reaction as described in Materials and Methods and were exposed to RP-Royal Xomat X-ray films for 6 h for hybridization between homologous DNAs and for 24 h for the hybridizations between heterologous DNAs. The effective temperature for hybridization in each case was determined by the melting temperature (T_m) of HPV-1 DNA at each formamide concentration, which was calculated from the equation T_m (°C) = 81.5 + 16.6 (log M) + 0.41 (% G+C) − 0.72 (% formamide), where M is the molarity of monovalent salt, and % G+C is the percentage of guanine plus cytosine residues in the DNA (18, 30). The % G+C for HPV-1 is 41% (4, 20).*

The presence of common sequences between the genomes of HPV-1 and BPV-2 was confirmed by a reciprocal experiment in which ^{32}P-labeled HPV-1 DNA was annealed to the four *HincII* fragments of BPV-2 under a variety of conditions. Again, no homology was detectable under the most stringent hybridization conditions utilized (effective temperature T_m − 30°C for BPV-2 DNA). In 40% formamide, however, stable hybrids formed between the ^{32}P-labeled HPV-1 probe and the BPV-2 *HincII* B fragment (Fig. 3). In 20 and 30% formamide stable hybrids

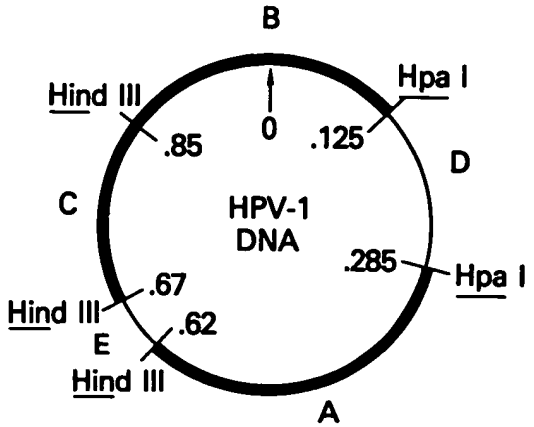

FIG. 2. *Circular physical map of human papilloma virus type 1 DNA (6). The five HpaI/HindIII cleavage fragments are labeled according to their size. The A, B, and C fragments containing sequences homologous to bovine papillomavirus type 2 DNA are indicated in bold lines.*

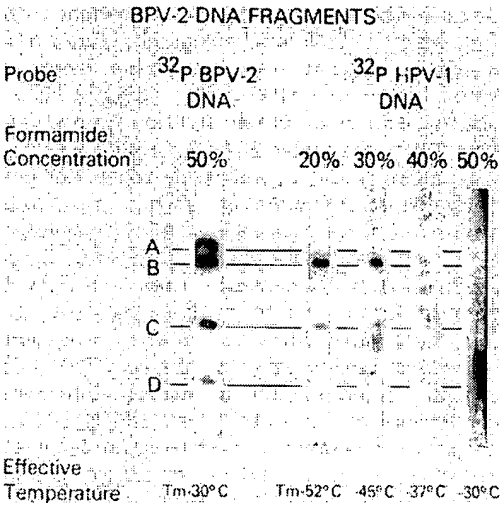

FIG. 3. *Hybridization of ^{32}P-labeled human papillomavirus type 1 (HPV-1) DNA to fragments of the bovine papillomavirus type 2 (BPV-2) genome. Nitrocellulose filter strips (5 mm wide) containing unlabeled HincII fragments of the BPV-2 genome (0.05 μg per strip) were incubated for 18 h in 1 ml of reaction mixture containing 500,000 cpm of in vitro ^{32}P-labeled and denatured HPV-1 DNA or BPV-2 DNA (specific activities were 7.0 × 10^7 cpm/μg and 9.0 × 10^7 cpm/μg, respectively) at 35°C with the indicated formamide concentration. The strips were processed as described in the legend of Fig. 1. The effective temperature at each formamide concentration was determined from the T_m calculated from the equation in the legend of Fig. 1. The % G+C for BPV-2 is 45.5 (4).*

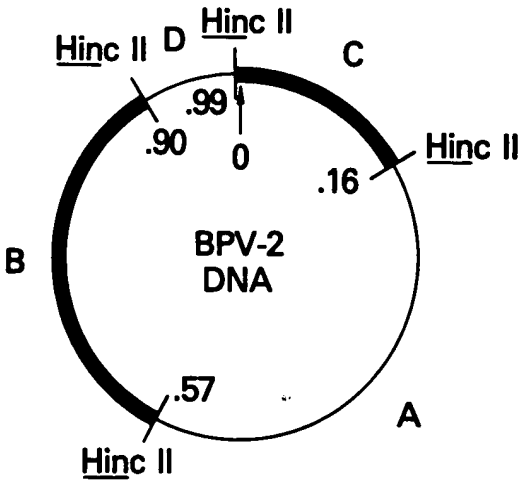

FIG. 4. *Circular physical map of the bovine papillomavirus type 2 genome (W. D. Lancaster, manuscript submitted for publication). The four HincII fragments are labeled according to their size; the single HindIII cleavage site is used as the 0 map position. The B and C fragments containing sequences homologous to human papillomavirus type 1 are indicated in bold lines.*

were detected only with BPV-2 *Hinc*II fragments B and C. Thus, sequences homologous to HPV-1 DNA with a maximum of approximately 25% base mismatch are located in the BPV-2 *Hinc*II B fragment and homologous sequences with up to 30% base mismatch are located in the BPV-2 *Hinc*II B and C fragments. The location of the four *Hinc*II fragments of BPV-2 are indicated in Fig. 4 (W. D. Lancaster, manuscript submitted for publication); the fragments containing the homology detected with HPV-1 are indicated with the bold lines; and, as in the HPV-1 genome, the homologous segments are noncontiguous.

Hybridization of BPV-1 and CRPV DNAs to fragments of the HPV-1 and BPV-2 genomes. To further examine the sequence homology among the genomes of various papillomaviruses, radiolabeled probes prepared in vitro from BPV-1 and CRPV DNAs were annealed to the HPV-1 *Hind*III/*Hpa*I DNA fragments and the BPV-2 *Hinc*II DNA fragments. In 50% formamide no homology was detectable between ^{32}P-labeled BPV-1 DNA and HPV-1 DNA fragments. In 40% formamide hybridization of the ^{32}P-labeled BPV-1 DNA probe to HPV-1 fragment A was detected, and in 30% formamide specific annealing to fragments A, B, and C was detected (Fig. 5). No annealing of the BPV-1 DNA probe to HPV-1 fragments D or E was detected even in 20% formamide. Thus, by this analysis the fragments of HPV-1 DNA containing regions of homology with BPV-1 DNA are the same fragments containing the regions of homology with BPV-2 DNA, and the effective temperatures at which the specific regions of homology melt are identical.

Homology was also detectable between the genomes of HPV-1 and CRPV. Although no stable hybrids were detectable between a CRPV probe and any of the HPV-1 *Hind*III/*Hpa*I fragments in 40% formamide, stable hybrids were formed in 30% formamide with the HPV-1 fragments A, B, and C (Fig. 5). In addition, the CRPV probe formed a stable hybrid with the HPV-1 D fragment (Fig. 5) in 20% formamide and faintly in 25% formamide. Thus, a region of homology with approximately 35% base mismatch mapping in the HPV-1 D fragment can be detected between the CRPV and HPV-1 genomes which is not shared between the HPV-1 DNA and the genomes of the bovine papillomaviruses.

^{32}P-labeled CRPV DNA did not form any stable hybrids with any of the BPV-2 *Hinc*II

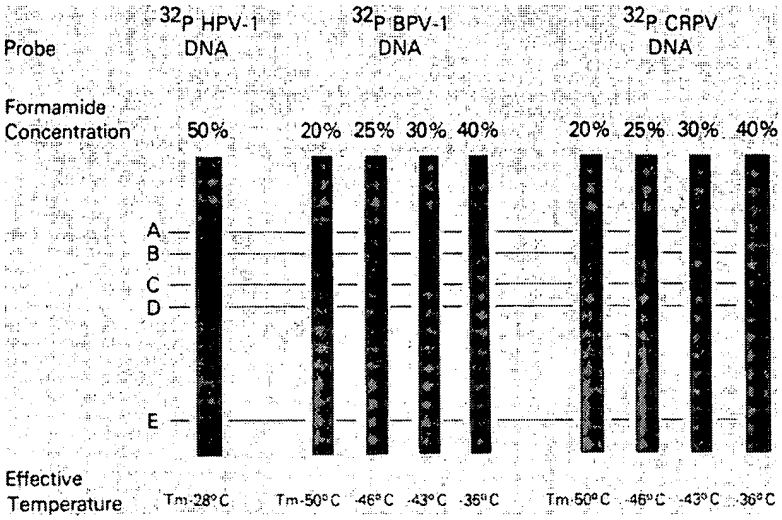

FIG. 5. *Hybridization of ^{32}P-labeled bovine papillomavirus type 1 (BPV-1) or cottontail rabbit papillomavirus (CRPV) DNA to fragments of the human papillomavirus type 1 (HPV-1) genome. Nitrocellulose filter strips containing HindIII/HpaI fragments of the HPV-1 genome (0.05 μg per strip) were incubated for 18 h in 1 ml of reaction mixture containing in vitro ^{32}P-labeled and denatured HPV-1 (specific activity, 1.2 × 10^8 cpm/μg), BPV-1 (specific activity, 8 × 10^7 cpm/μg) or CRPV (specific activity, 6 × 10^7 cpm/μg) DNA at 35°C at the indicated formamide concentration. The strips were processed as described in the legend of Fig. 1.*

fragments in 40% formamide; however, it did form stable hybrids with *Hin*cII fragments B and C in 20, 25, and 30% formamide (Fig. 6). Thus, at the level of 30% base mismatch CRPV shares homology with the BPV-2 genome in the same BPV-2 fragments which share homology with HPV-1 DNA (Fig. 3).

^{32}P-labeled BPV-1 DNA formed stable hybrids with the BPV-2 DNA fragments even in 60% formamide (Fig. 6). This result is in good agreement with the findings of Lancaster and Olson that the BPV-1 and BPV-2 genomes are up to 58% homologous under standard stringent conditions of hybridization (14). These two viruses are clearly closely related. The results presented here indicate that the strong regions of homology are not localized in one portion of the genome but rather are dispersed throughout the genome and are represented in each of the four *Hin*cII fragments. Similar results were obtained when the isolated labeled *Hin*cII BPV-2 fragments were hybridized to BPV-1 DNA under stringent conditions (W. D. Lancaster, unpublished data).

Competitive hybridization of ^{32}P-labeled CRPV DNA to BPV-2 DNA by unlabeled HPV-1 DNA. To determine whether the homologous sequences detected between the BPV-2 genome and CRPV DNA were the same as those sequences shared by BPV-2 and HPV-1 DNA, we performed a competition hybridization experiment. In vitro ^{32}P-labeled CRPV DNA was incubated with nitrocellulose strips containing the four *Hin*cII fragments of BPV-2 DNA in 20% formamide and different concentrations of unlabeled HPV-1 DNA for 18 h (Fig. 7). As expected, ^{32}P-labeled CRPV DNA formed stable hybrids with BPV-2 DNA fragments B and C when no HPV-1 DNA was added to the hybridization mixture. Although the addition of 0.5 μg of unlabeled HPV-1 DNA had little if any detectable effect on the annealing of ^{32}P-labeled CRPV DNA, the presence of 5.0 μg of HPV-1 DNA completely blocked its specific hybridization (Fig. 7). This indicates that the sequences in the BPV-2 *Hin*cII B and C fragments homologous with CRPV DNA are the same as those sequences shared by HPV-1 DNA and BPV-2 DNA. The presence of common sequences among three papillomaviruses whose natural hosts are so different strongly suggests that these sequences are conserved among the genomes of all papillomaviruses.

Lack of homology between the papillomaviruses and the polyoma viruses. We extended these studies to examine the relationship of the *Papillomavirus* genus to the *Polyomavirus* genus. ^{32}P-labeled HPV-1 DNA was hybridized to specific restriction endonuclease fragments of SV40 and BK(MM) DNAs immobilized on nitrocellulose filters. Even in 20% formamide (low-stringency conditions under which the homology between SV40 and polyoma virus DNAs can be demonstrated) (12), no stable hybrids

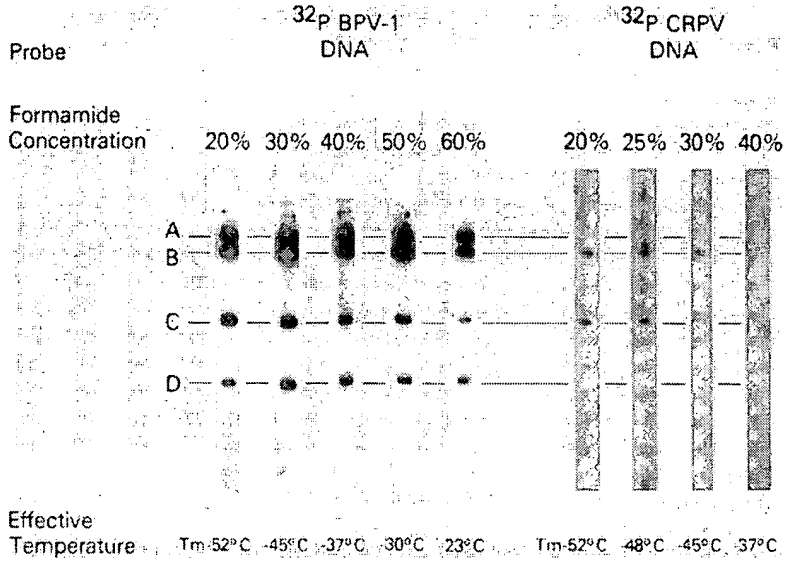

FIG. 6. *Hybridization of ^{32}P-labeled bovine papillomavirus type 1 (BPV-1) or cottontail rabbit papillomavirus (CRPV) DNA to fragments of the bovine papillomavirus type 2 (BPV-2) genome. Nitrocellulose filter strips containing HincII fragments of the BPV-2 genome (0.05 μg per strip) were incubated for 18 h in 1 ml of a reaction mixture containing 500,000 cpm of in vitro ^{32}P-labeled and denatured BPV-1 (specific activity, 8 × 10^7 cpm/μg) or CRPV (specific activity, 6 × 10^7 cpm/μg) DNA at 35°C in the indicated formamide concentrations. The strips were processed as described in the legend of Fig. 1.*

could be detected between HPV-1 DNA and either SV40 or BK(MM) DNA fragments (Fig. 8). Although the data are not shown, similar negative results were obtained when radiolabeled SV40 or BK(MM) DNAs were annealed with immobilized HPV-1 *Hin*dIII/*Hpa*I fragments.

DISCUSSION

In this study we have demonstrated the presence of conserved nucleotide sequences among the genomes of several papillomaviruses. Since these regions of homology contain 25 to 35% base mismatch they cannot be detected by DNA reassociation kinetics under standard hybridization conditions at T_m − 25°C. However, under the less stringent conditions employed in this study, these homologous regions formed stable heteroduplexes and could be detected at T_m − 43°C. The conserved homologous sequences were localized in the HPV-1 DNA *Hin*dIII/*Hpa*I A, B, and C fragments and in the BPV-2 DNA *Hin*cII B and C fragments. Since there is no information on the genetic organization of papillomavirus genomes, the localization of the conserved sequences to specific genes or functional regions of the papillomaviruses is not yet possible.

The regions of conserved homology among papillomaviruses appear to be genus or subgroup specific. No stable hybrids could be detected between the DNAs of HPV-1 and either of the primate polyoma viruses SV40 or BK(MM). It also appears from our studies that there is extensive homology between the genomes of the two bovine papillomaviruses, evidenced both by the even distribution of the homologous regions throughout the BPV-1 and BPV-2 genomes and by the low degree of base mismatch (at most 16.5%) within the homology regions (stable heteroduplexes were formed between the two genomes even at T_m − 23°C). The DNAs of the two bovine papillomaviruses also showed a similar hybridization pattern with HPV-1 DNA, in that they each shared homology with the HPV-1 DNA *Hin*dIII/*Hpa*I fragments A, B, and C, with the strongest homology in fragment A.

In addition to these conserved sequences, CRPV DNA contained a region of weak homology with HPV-1 DNA in the *Hin*dIII/*Hpa*I fragment D which was not detectable between BPV DNAs and the HPV-1 genome. This additional homology suggests that HPV-1 and CRPV are more closely related to each other than they are to the bovine papillomaviruses. This closer relationship may be reflected in the nature of the lesions produced by HPV-1 and CRPV, which are predominantly epithelial, in contrast to those produced by BPV-1 and BPV-2, which are fibro-epithelial.

Immunodiffusion experiments failed to show any antigenic similarities among virions of the

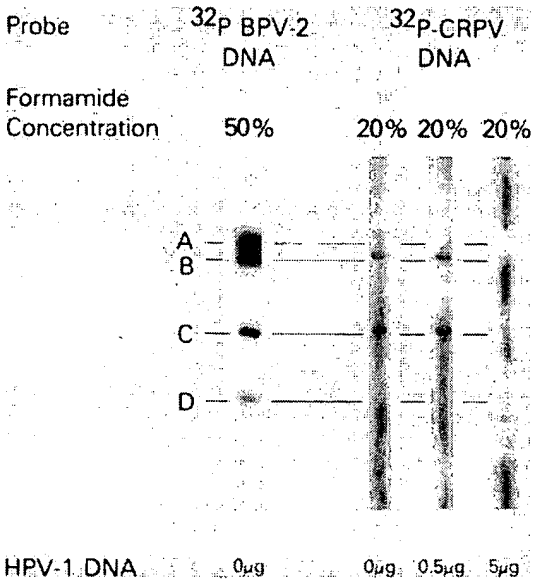

FIG. 7. *Competitive inhibition of the hybridization between ^{32}P-labeled cottontail rabbit papillomavirus (CRPV) DNA and fragments of the bovine papillomavirus type 2 (BPV-2) genome by unlabeled human papillomavirus type 1 (HPV-1) DNA. Nitrocellulose filter strips containing HincII fragments of the BPV-2 genome (0.05 μg per strip) were allowed to hybridize with 500,000 cpm of ^{32}P-labeled and denatured CRPV DNA (specific activity, 6×10^7 cpm/μg) in a reaction mixture containing 20% formamide and the indicated amount of unlabeled, sheared, and denatured HPV-1 DNA. Incubation was at 35°C for 18 h. The strips were processed as described in the legend of Fig. 1.*

papillomaviruses of different species (17). Recently, however, Jenson and co-workers have demonstrated that antisera against sodium dodecyl sulfate-disrupted human papillomavirus virions can cross-react with virus-positive papillomas of different species (B. Jenson, W. D. Lancaster, and K. Shah, personal communication). Independently, Orth et al. have also shown common antigenic determinants between HPV-1 and CRPV, using either sera of rabbits bearing transplanted CRPV-induced carcinomas or hyperimmune sera raised against either alkali-disrupted HPV-1 virions or against the main HPV-1 structural polypeptide (22). Presumably, these cross-reacting antigenic determinants are masked in intact viral particles since antisera prepared against intact HPV-1 virions reacted only with HPV-1 infected cells. A similar situation has also been observed for the different members of the polyoma virus subgroup, where a genus-specific antigen has been detected by using antisera against disrupted virions but not with intact virions (31). In addition, each of the antisera raised against disrupted virions of either HPV-1 or SV40 failed to cross-react with cells infected by viruses of the heterologous genus, indicating that the antigens detected are genus specific (P. M. Howley, unpublished data; B. Jenson, W. D. Lancaster, and K. Shah, personal communication).

Determining the precise location and size of the conserved sequences among the papillomaviruses requires additional analysis by either electron microscopic heteroduplex mapping (9) or hybridization to other genomic fragments generated by different restriction endonucleases. It is clear from the data presented here that the sequences common to the bovine papillomaviruses, HPV-1, and CRPV are localized in noncontiguous regions of the genome, indicating that the homology most likely exists in at least two different gene regions. Furthermore, the presence of a common genus-specific internal antigen indicates that at least one of the areas of conserved nucleotide sequences must encode a portion of a major or minor capsid protein. Since nothing is known of the genetic organization of the papillomavirus genomes, however, the identification of the actual genes containing the conserved sequences will require the elucidation of this organization.

The presence of common sequences among HPV-1, CRPV, BPV-1, and BPV-2 DNAs indicates that a closer evaluation of the potential role of the human papillomaviruses in human neoplasia may be warranted. The oncogenic potentials of the bovine papillomaviruses and CRPV have been well established. CRPV is capable of producing carcinomas as well as benign skin papillomas in its natural host, the cottontail rabbit, as well as in the domestic rabbit (13, 34). Experimentally, the bovine papillomaviruses are capable of producing meningiomas and polypoid bladder tumors in their natural host and benign as well as malignant stromal tumors in alien hosts such as mice or hamsters (21). One of the human papillomaviruses, HPV-5, may have oncogenic potential in humans in that verrucous lesions containing HPV-5 in patients with epidermodysplasia verruciformis appear to undergo malignant conversion to squamous cell carcinoma rather frequently (23). The systematic screening of human tumors for papillomavirus sequences has not been feasible because of the increasing number of human papillomaviruses whose DNAs do not cross-hybridize under stringent conditions. However, because of the common DNA sequences among the papillomaviruses demonstrated in this manuscript, it should be possible to assay for papillomavirus-specific DNA sequences in

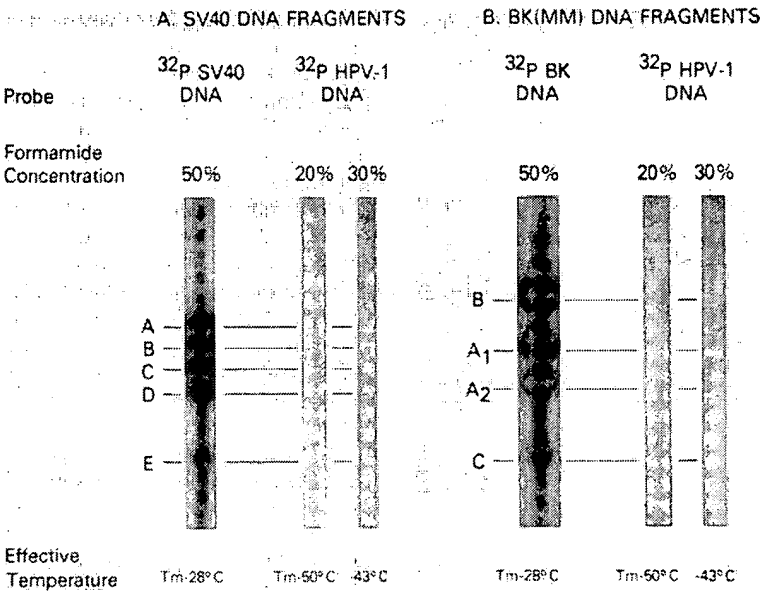

FIG. 8. *Hybridization of ^{32}P-labeled HPV-1 DNA to the fragments of (A) the simian virus 40 (SV40) or (B) the MM isolate of human papovavirus BK [BK(MM)] genomes. Nitrocellulose filter strips containing unlabeled EcoRI/BglI/HpaI fragments of the SV40 genome, or unlabeled BamHI/HindIII fragments of the BK(MM) genome, were allowed to hybridize to ^{32}P-labeled and denatured homologous DNA or human papillomavirus type 1 (HPV-1) DNA under the conditions indicated and were processed as described in the legend of Fig. 1.*

human tumors under less stringent conditions (i.e., T_m − 43°C) using any one of the human papillomavirus DNAs as a probe.

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ticular agency. *Id.* at 83. The decision to reallocate Treasury as stated in PS251 was nothing more than contract administration undertaken to produce the revenue targets stated by the government's concept paper. *Id.* at 84.

AT&T achieved 94% of its *target* 76% revenue share. As previously discussed, AT&T's revenue share was a potential target, not a guarantee. Moreover, AT&T conceded that it might not achieve the full 76% when it signed the Service Level Agreement that permitted the government to suspend further transfers based on AT&T's unacceptable performance. The government also points out that AT&T obtained other benefits, including the benefit of taking business away from Sprint, as opposed to losing 40% of its business to Sprint, and the benefit of providing telecommunication services to the government in exchange for billions of dollars. The Board found that AT&T also benefited from a "positive positioning in the industry," "the ability to serve new customers," and the competitive advantage "in any new competition to replace the FTS2000 contract." *Board's Opinion* at 30. We therefore disagree with AT&T that the government has received benefits that are "grossly disproportional" to the benefits conferred on AT&T by the contract.

III.

We agree with the Board that the government did not breach or repudiate its contract with AT&T, nor do we find that the government has been unjustly enriched. The Board's decision denying AT&T restitution is therefore affirmed.

AFFIRMED.

ENZO BIOCHEM, INC.,
Plaintiff-Appellant,

v.

GEN-PROBE INCORPORATED,

and

Chugai Pharma U.S.A., Inc. and Chugai Pharmaceutical Co., Ltd.,

and

Biomerieux, Inc.,

and

Becton Dickinson and Company,
Defendants-Appellees,

and

Biomerieux SA, Defendant.

No. 01-1230.

United States Court of Appeals, Federal Circuit.

DECIDED: July 15, 2002.

Assignee of patent directed to nucleic acid probes that selectively hybridize to genetic material of bacteria that cause gonorrhea brought patent infringement suit against competitors, who moved for summary judgment. The United States District Court for the Southern District of New York, Alvin K. Hellerstein, J., granted motion. Assignee appealed. On grant of petition for rehearing, the Court of Appeals, Lourie, Circuit Judge, held that: (1) patent's reference to deposit in public depository can constitute adequate description of claimed material for purpose of written description requirement; (2) fact issues existed as to whether one skilled in the art would view various subsequences, mutations, and mixtures of deposited sequences as within scope of claims; (3) fact issues existed as to whether deposited sequences were representative of broader genus claims; (4) fact issues existed as to whether claimed sequences were adequately described in terms of function; and (5) specification's indication that assignee possessed claimed invention by reducing it to practice was insufficient alone to meet written description requirement.

Reversed and remanded.

Opinion, 285 F.3d 1013, vacated.

1. Patents ⇐112.5

A patent is presumed to be valid, and this presumption can be overcome only by facts supported by clear and convincing evidence to the contrary. 35 U.S.C.A. § 282.

2. Patents ⇐314(5)

Compliance with the patent statute's written description requirement is a question of fact. 35 U.S.C.A. § 112.

3. Patents ⇐99

Written description requirement of patent statute calls for a written description of an invention separate from enablement. 35 U.S.C.A. § 112.

4. Patents ⇐99

Compliance with the patent statute's written description requirement is essentially a fact-based inquiry that will necessarily vary depending on the nature of the invention claimed. 35 U.S.C.A. § 112.

5. Patents ⇐97

Regulatory guidelines governing internal practice of Patent and Trademark Office (PTO) for examining patent applications under statutory written description requirement, like the Manual of Patent Examining Procedure (MPEP), are not binding on Court of Appeals, but may be given judicial notice to the extent they do not conflict with the statute. 35 U.S.C.A. § 112.

6. Patents ⇐99

Patent statute's written description requirement can be met by showing that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics. 35 U.S.C.A. § 112.

7. Patents ⇐99

Reference in patent specification to deposits of claimed nucleotide sequences in public depository sufficiently described those sequences to the public for purposes of patent statute's written description requirement; a person of skill in the art, reading the accession numbers in the patent specification, could obtain claimed sequences from depository by following the appropriate techniques to excise the nucleotide sequences from the deposited organisms containing those sequences, and, although structures of those sequences were not expressly set forth in the specification, those structures may not have been reasonably obtainable and in any event were not known to patent applicant when application was filed. 35 U.S.C.A. § 112.

8. Patents ⇐99

Reference in patent specification to a deposit in a public depository, which

makes its contents accessible to the public when it is not otherwise available in written form, constitutes an adequate description of the deposited material sufficient to comply with the patent statute's written description requirement. 35 U.S.C.A. § 112.

9. Federal Civil Procedure ⇐2508

Genuine issue of material fact as to whether three claimed nucleotide sequences placed in public depository also described various subsequences, mutations, and mixtures of those sequences, which allegedly also fell within scope of patent claims, to one skilled in the art precluded summary judgment for alleged infringer, who asserted that such substantial breadth of claims would render them invalid under the written description requirement. 35 U.S.C.A. § 112.

10. Federal Civil Procedure ⇐2508

Genuine issue of material fact as to whether three claimed nucleotide sequences placed in public depository were representative, to one skilled in the art, of broad genus claims, in patent directed to nucleic acid probes that selectively hybridize to the genetic material of the bacteria that cause gonorrhea, precluded summary judgment for alleged infringer on its claim that genus claims were invalid for failure to meet patent statute's written description requirement. 35 U.S.C.A. § 112.

11. Federal Civil Procedure ⇐2508

Genuine issue of material fact as to whether disclosed correlation of their function of hybridization with bacterial DNA strains deposited in public depository described claimed nucleotide sequences, in patent directed to nucleic acid probes that selectively hybridized to genetic material of bacteria that caused gonorrhea, even though DNA structures were not explicitly sequenced, precluded summary judgment for alleged infringer on its claim that patent was invalid for failure to meet the written description requirement. 35 U.S.C.A. § 112.

12. Patents ⇐99

Fact that claims of patent directed to nucleic acid probes that selectively hybridize to the genetic material of the bacteria that cause gonorrhea appeared in ipsis verbis in the written description did not automatically satisfy written description requirement of patent statute. 35 U.S.C.A. § 112.

13. Patents ⇐99

Even if a patent claim is supported by the specification, the language of the specification, to the extent possible, must describe the claimed invention so that one skilled in the art can recognize what is claimed; the appearance of mere indistinct words in a specification or a claim, even an original claim, does not necessarily satisfy that requirement. 35 U.S.C.A. § 112.

14. Patents ⇐99

A description of what a material does, rather than of what it is, usually does not suffice to meet the patent statute's written description requirement; disclosure must allow one skilled in the art to visualize or recognize the identity of the subject matter purportedly described. 35 U.S.C.A. § 112.

15. Patents ⇐99

Where the words of the patent claim alone do not convey an adequate description of the invention, regardless of whether the claim appears in the original specification and is thus supported by the specification as of the filing date, the patent statute's written description requirement is not necessarily met. 35 U.S.C.A. § 112.

16. Patents ⇐99

If a purported description of an invention does not meet patent statute's written description requirement, the fact that it appears as an original claim or in the specification does not save it; a claim does not become more descriptive by its repetition, or its longevity. 35 U.S.C.A. § 112.

17. Patents ⇐99

Mere fact that specification of patent, which was directed to nucleic acid probes that selectively hybridize to the genetic material of the bacteria that cause gonorrhea, indicated that patent holder possessed claimed invention by reducing to practice three nucleotide sequences within the scope of the patent claims and depositing them in public depository did not establish that patent met the statutory written description requirement. 35 U.S.C.A. § 112.

18. Patents ⇐99

While articulation of written description requirement of patent statute in terms of "possession" is useful when a patentee is claiming entitlement to an earlier filing date, in interferences in which the issue is whether a count is supported by the specification of one or more of the parties, and in ex parte applications in which a claim at issue was filed subsequent to the application, application of written description requirement is not subsumed by the "possession" inquiry. 35 U.S.C.A. § 112.

19. Patents ⇐99

A showing that the patentee is in "possession" of the claimed invention is ancillary to the statutory mandate of an adequate written description, and that requirement is not met if, despite a showing of possession, the specification does not adequately describe the claimed invention. 35 U.S.C.A. § 112.

20. Patents ⇐99

Although one can show possession of an invention by means of an affidavit or declaration during prosecution, as one does in an interference or when one files an affidavit to antedate a reference, such a showing of possession does not substitute for a written description in the specification, as required by patent statute. 35 U.S.C.A. § 112.

21. Patents ⇐99

Proof of a reduction to practice, absent an adequate description in the specification of what is reduced to practice, does not serve to describe or identify the invention for purposes of the patent statute's written description requirement. 35 U.S.C.A. § 112.

22. Patents ⇐99

Written description requirement is the quid pro quo of the patent system; the public must receive meaningful disclosure in exchange for being excluded from practicing the invention for a limited period of time. 35 U.S.C.A. § 112.

Richard L. Delucia, Kenyon & Kenyon, of New York, NY, filed a petition for rehearing en banc for plaintiff-appellant. With him on the petition were Charles A. Weiss and Bradley S. Corsello.

The appellees filed a consolidated response to the petition for rehearing en banc. William F. Lee, Hale and Dorr

LLP, of Boston, MA, for defendant-appellee Gen-Probe Incorporated. With him on the response was William G. McElwain.

Robert J. Gunther, Jr., Latham & Watkins, of New York, NY, for defendants-appellees Chugai Pharma U.S.A., Inc. and Chugai Pharmaceutical Co., Ltd. With him on the response was Jeffrey A. Tochner. Of counsel was Kurt M. Rogers.

Daniel A. Boehnen, McDonnell Boehnen Hulbert & Berghoff, of Chicago, IL, for defendant-appellee Biomerieux, Inc. With him on the response was Joshua R. Rich.

Donald R. Ware, Foley Hoag & Eliot LLP, of Boston, MA, for defendant-appellee Becton Dickinson and Company. With him on the response was Barbara A. Fiacco.

Frank P. Porcelli, Fish & Richardson P.C., of Boston, MA, filed a brief for amicus curiae Fish & Richardson P.C. Of counsel on the brief were Robert E. Hillman and Charles H. Sanders.

Mark S. Davies, Attorney, Appellate Staff, Civil Division, Department of Justice, of Washington, DC, filed an amicus curiae brief for the United States in support of rehearing en banc. With him on the brief were Robert D. McCallum, Jr., Assistant Attorney General, and Scott R. McIntosh, Attorney. Of counsel on the brief was John M. Whealan, Solicitor, U.S. Patent and Trademark Office, of Arlington, Virginia.

Before LOURIE, DYK and PROST, Circuit Judges.

ON PETITION FOR REHEARING

LOURIE, Circuit Judge.

Enzo Biochem, Inc. petitions for rehearing of this appeal following our prior decision, reported at 285 F.3d 1013, 62 USPQ2d 1289 (Fed.Cir.2002), in which we affirmed the decision of the United States District Court for the Southern District of New York. The district court had granted Gen-Probe Incorporated, Chugai Pharma U.S.A., Inc., Chugai Pharmaceutical Co., Ltd., Biomerieux, Inc., Biomerieux SA, and Becton Dickinson and Company's (collectively, "the defendants") motion for summary judgment that claims 1–6 of U.S. Patent 4,900,659 are invalid for failure to meet the written description requirement of 35 U.S.C. § 112, ¶ 1. *Enzo Biochem, Inc. v. Gen-Probe Inc.*, No. 99 Civ. 4548 (S.D.N.Y. Apr. 4, 2001) (final order). Having considered Enzo's petition for rehearing and the defendants' response,[1] we have determined that our prior decision that a deposit may not satisfy the written description requirement was incorrect. We therefore grant Enzo's petition for rehearing, vacate the prior decision, and reverse the district court's grant of summary judgment that Enzo's claims are invalid for failure to meet the written description requirement. Because genuine issues of material fact exist regarding satisfaction of the written description requirement, we remand.

1. *Amicus curiae* briefs were filed by the United States Patent and Trademark Office and Fish & Richardson P.C.

BACKGROUND

Enzo is the assignee of the '659 patent, which is directed to nucleic acid probes that selectively hybridize to the genetic material of the bacteria that cause gonorrhea, *Neisseria gonorrhoeae*. *N. gonorrhoeae* reportedly has between eighty and ninety-three percent homology with *Neisseria meningitidis*. '659 patent, col. 2, ll. 61–64. Such a high degree of homology has made detection of *N. gonorrhoeae* difficult, as any probe capable of detecting *N. gonorrhoeae* may also show a positive result when only *N. meningitidis* is present. Enzo recognized the need for a chromosomal DNA probe specific for *N. gonorrhoeae*, and it derived three such sequences that preferentially hybridized to six common strains of *N. gonorrhoeae* over six common strains of *N. meningitidis*. *Id.* at col. 3, l. 49 to col. 4, l. 14; col. 4, ll. 45–50. The inventors believed that if the preferential hybridization ratio of *N. gonorrhoeae* to *N. meningitidis* were greater than about five to one, then the "discrete nucleotide sequence [would] hybridize to virtually all strains of *Neisseria gonorrhoeae* and to no strain of *Neisseria meningitidis*." *Id.* at col. 12, ll. 60–65. The three sequences that the inventors actually derived had a selective hybridization ratio of greater than fifty. *Id.* at col. 13, ll. 9–15. Enzo deposited those sequences in the form of a recombinant DNA molecule within an *E. coli* bacterial host at the American Type Culture Collection. *Id.* at col. 13, ll. 27–31.

Claim 1 is as follows:

1. A composition of matter that is specific for *Neisseria gonorrhoeae* comprising at least one nucleotide sequence *for which the ratio of the amount of said sequence which hybridizes to chromosomal DNA of Neisseria gonorrhoeae to the amount of said sequence which hybridizes to chromosomal DNA of Neisseria meningitidis is greater than about five*, said ratio being obtained by a method comprising the following steps;

(a) providing a radioactively labeled form of said nucleotide sequence;

(b) providing a serial dilution series of purified chromosomal DNA from each of the *N. gonorrhoeae* strains; (1) ATCC 53420, (2) ATCC 53421, (3) ATCC 53422, (4) ATCC 53423, (5) ATCC 53424, (6) ATCC 53425, and forming test dots from each of said dilution series on a matrix;

(c) providing a serial dilution series of purified nucleotide sequences from each of the *N. meningitidis* strains: (1) ATCC 53414, (2) ATCC 53415, (3) ATCC 53416, (4) ATCC 53417, (5) ATCC 53418, (6) ATCC 53419, and forming test dots from each of said dilution series on a matrix;

(d) hybridizing equal portions of the labeled nucleotide sequences to the matrix provided in step (b) and (c), respectively; wherein the hybridization is conducted in a solution having a salt concentration of 2X SSC at (i) 65°C. in cases in which the sequence has greater than 50 base pairs or (ii) at Tm (°C.) minus 30°C. in cases in which the sequence has less than 50 base pairs, wherein Tm is the denaturation temperature of the sequence;

(e) quantifying the labeled nucleotide sequence hybridized in step (d) to each test dot;

(f) subtracting from the data of step (e) an averaged amount of radioactivity attributable to background to obtain a corrected amount of hybridized radioactivity at each test dot;

(g) normalizing the data of step (f) by multiplying the amount of corrected radioactivity at each test dot by a factor which adjusts the amount of radioactivity to equal amounts of chromosomal DNA at each test dot;

(h) selecting two normalized values that are most nearly the same and that correspond to adjacent members

of the dilution series for each of the above strains of *N. gonorrhoeae* and obtaining the average of the selected values;

(i) selecting two normalized values that are most nearly the same and that correspond to adjacent members of the dilution series for each of the above strains of *N. meningitidis* and obtaining the average of the selected values;

(j) dividing the lowest average obtained in step (h) by the highest average obtained in step (i) to obtain said ratio.

Id. at col. 27, l. 29 to col. 28, l. 27 (emphasis added). Claims 2 and 3 depend from claim 1 and further limit the hybridization ratio to greater than about twenty-five and fifty, respectively. *Id.* at col. 2, ll. 27–30. Claim 4 is directed to the three deposited sequences (referenced by their accession numbers) and variants thereof as follows:

4. The composition of claim 1 wherein said nucleotide sequences are selected from the group consisting of:

a. the *Neisseria gonorroheae* [sic] DNA insert of ATCC 53409, ATCC 53410 and ATCC 53411, and discrete nucleotide subsequences thereof,

b. mutated discrete nucleotide sequences of any of the foregoing inserts that are within said hybridization ratio and subsequences thereof; and

c. mixtures thereof.

Id. at col. 28, ll. 31–39. Claim 5 is directed to an assay for detection of *N. gonorrhoeae* using the composition of claim 1. *Id.* at ll. 40–46. Claim 6 further limits the method of claim 5 to the nucleotide sequences that Enzo deposited (*i.e.*, those in claim 4) and variants thereof. *Id.* at ll. 47–56.

Enzo sued the defendants for infringement of the '659 patent, and the defendants moved for summary judgment that the claims were invalid for failure to meet the written description requirement of 35 U.S.C. § 112, ¶ 1. The district court, in oral remarks from the bench, granted that motion. Tr. of Hr'g at 42, *Enzo Biochem, Inc. v. Gen-Probe, Inc.*, No. 99-CV-4548 (S.D.N.Y. Jan. 24, 2001). It concluded that the claimed composition of matter was defined only by its biological activity or function, *viz.*, the ability to hybridize to *N. gonorrhoeae* in a ratio of better than about five with respect to *N. meningitidis*, which it was held was insufficient to satisfy the § 112, ¶ 1 requirement set forth in this court's holdings in *Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 43 USPQ2d 1398 (Fed.Cir. 1997), *Fiers v. Revel*, 984 F.2d 1164, 25 USPQ2d 1601 (Fed.Cir.1993), and *Amgen, Inc. v. Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 USPQ2d 1016 (Fed.Cir. 1991). Tr. of Hr'g at 28. The court rejected Enzo's argument that the reference in the specification to the deposits of biological materials in a public depository inherently disclosed that the inventors were in possession of the claimed sequences. *Id.* at 35. It distinguished this court's precedents concerning deposits as relating to the enablement requirement of § 112, ¶ 1. *Id.* at 38–40. Enzo appealed to this court; we have jurisdiction pursuant to 28 U.S.C. § 1295(a)(1).

DISCUSSION

[1, 2] Summary judgment is appropriate when there is no genuine issue of material fact and the moving party is entitled to judgment as a matter of law. Fed. R.Civ.P. 56(c); *Anderson v. Liberty Lobby, Inc.*, 477 U.S. 242, 247–48, 106 S.Ct. 2505, 91 L.Ed.2d 202 (1986). On motion for summary judgment, the court views the evidence and any disputed factual issues in the light most favorable to the party opposing the motion. *Matsushita Elec. Indus. Co. v. Zenith Radio Corp.*, 475 U.S. 574, 587, 106 S.Ct. 1348, 89 L.Ed.2d 538 (1986). A patent is presumed to be valid, 35 U.S.C. § 282 (1994), and this presumption can be overcome only by facts supported by clear and convincing evidence to the contrary, *see, e.g., WMS Gaming, Inc. v. Int'l Game Tech.*, 184 F.3d 1339, 1355, 51 USPQ2d 1385, 1396–97 (Fed.Cir.1999). Compliance with the written description requirement is a question of fact. *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563, 19 USPQ2d 1111, 1116 (Fed.Cir. 1991).

Enzo argues that the testimony of its expert, Dr. Wetmer, raised a genuine factual issue whether the reference to the deposits inherently described the claimed nucleotide sequences. Enzo also argues that its description of the binding affinity of the claimed nucleotide sequences satisfies the requirement set forth in the Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 "Written Description" Requirement, 66 Fed. Reg. 1099 (Jan. 5, 2001) ("*Guidelines*"). Enzo asserts that the court erred in not evaluating the patentability of the claims separately, pointing out that claims 4 and 6 are directed to the three deposited sequences and variations and mixtures thereof. Enzo further asserts that the claims *per se* meet the written description requirement because they appear *in ipsis verbis* in the written description. Enzo also argues that this court's articulation of the written description requirement for genetic material in *Eli Lilly* should not apply to this case because Enzo reduced the invention to practice and deposited the derived biological materials, thereby demonstrating its "possession" of the invention.

The defendants respond that the district court properly granted summary judgment because the patent described the claimed nucleotide sequences only by their function, which they state is insufficient to meet the requirements of § 112, ¶ 1 as a matter of law, even as to the narrower claims directed to the deposited materials. The defendants also assert that Dr. Wetmur's opinion that the deposited genetic materials could have been sequenced did not cure the actual failure of the inventors to identify them by some distinguishing characteristic, such as their structure. Moreover, the defendants point out that claims 4 and 6, which are directed to the deposited materials, each cover a broad genus of nucleic acids. The defendants also urge that *in ipsis verbis* support for the claims in the specification does not *per se* establish compliance with the written description requirement. Finally, the defendants assert that the district court did not err in its determination that Enzo's "possession" of three nucleotide sequences that it reduced to practice and deposited nevertheless did not satisfy the written description requirement of § 112, ¶ 1.

[3, 4] The written description requirement of § 112, ¶ 1 is set forth as follows:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

scribe how to make and use the invention in a reproducible manner." MPEP § 2402 (8th ed. Aug. 2001). The PTO has also issued a regulation stating when a deposit is not necessary, *i.e.*, "if it is known and readily available to the public or can be made or isolated without undue experimentation." 37 C.F.R. § 1.802(b) (2001). Inventions that cannot reasonably be enabled by a description in written form in the specification, but that otherwise meet the requirements for patent protection, may be described in surrogate form by a deposit that is incorporated by reference into the specification. While deposit in a public depository most often has pertained to satisfaction of the enablement requirement, we have concluded that reference in the specification to a deposit may also satisfy the written description requirement with respect to a claimed material.

In this case, Enzo's deposits were incorporated by reference in the specification. A person of skill in the art, reading the accession numbers in the patent specification, can obtain the claimed sequences from the ATCC depository by following the appropriate techniques to excise the nucleotide sequences from the deposited organisms containing those sequences. '659 patent, col. 13, ll. 27–36. The sequences are thus accessible from the disclosure in the specification. Although the structures of those sequences, *i.e.*, the exact nucleotide base pairs, are not expressly set forth in the specification, those structures may not have been reasonably obtainable and in any event were not known to Enzo when it filed its application in 1986. *See* '659 patent, col. 3, ll. 40–46 (noting severe time constraints in sequencing DNA). We therefore agree with Enzo that reference in the specification to deposits of nucleotide sequences describe those sequences sufficiently to the public for purposes of meeting the written description requirement.

[9] As the defendants point out, however, Enzo's claims 4 and 6 are not limited to the deposited sequences. Claim 4 is directed to nucleotide sequences that are selected from the group consisting of the three deposited sequences, "discrete nucleotide subsequences thereof ... mutated discrete nucleotide sequences of any of the foregoing inserts that are within said hybridization ratio and subsequences thereof[,] and ... mixtures thereof." '659 patent, col. 28, ll. 31–39. Claim 6 is also similarly directed to the three deposited sequences and subsequences and mutated variations thereof. *Id.* at ll. 47–56. The specification defines a subsequence nonspecifically as a nucleotide sequence "greater than about 12 nucleotides." '659 patent, col. 3, ll. 29–30. As the deposited sequences are about 850, 850, and 1300 nucleotides long, *id.* at col. 13, ll. 47–49, there are at least hundreds of subsequences of the deposited sequences, an unknown number of which might also meet the claimed hybridization ratio. Moreover, Enzo's expert, Dr. Wetmur, stated that "astronomical" numbers of mutated variations of the deposited sequences also fall within the scope of those claims, and that such broad claim scope is necessary to adequately protect Enzo's invention from copyists who could otherwise make a minor change to the sequence and thereby avoid infringement while still exploiting the benefits of Enzo's invention. The defendants assert that such breadth is fatal to the adequacy of the written description. On the other hand, because the deposited sequences are described by virtue of a reference to their having been deposited, it may well be that various subsequences, mutations, and mixtures of those sequences are also described to one of skill in the art. We regard that question as an issue of fact that is best resolved on remand.[2] Because the district court's grant of summary judgment was based on its conclusion that Enzo's deposits could not satisfy the written description requirement as a matter of law, we reverse the district court's grant of summary judgment that claims 4 and 6 are invalid for failure to meet the written description requirement. On remand, the court should determine whether a person of skill in the art would glean from the written description, including information obtainable from the deposits of the claimed sequences, subsequences, mutated variants, and mixtures sufficient to demonstrate possession of the generic scope of the claims.

[10] We next address the question whether the compositions of the broader genus claims 1–3 and 5 are sufficiently described to meet the requirements of § 112, ¶ 1, on the basis of Enzo's deposits of three sequences. If those sequences are representative of the scope of the genus claims, *i.e.*, if they indicate that the patentee has invented species sufficient to constitute the genera, they may be representative of the scope of those claims. *See In re Smythe*, 480 F.2d 1376, 1383, 178 USPQ 279, 284–85 (CCPA 1973) (discussing circumstances in which a species may be representative of and therefore descriptive of genus claims). Because the district court concluded that the deposited sequences were not themselves described, it did not determine whether that description was representative of the genera in those claims. Such determination should be made on remand.

When we addressed a similar issue in *Eli Lilly*, we determined that a disclosure of the sequence of rat cDNA was not descriptive of the broader invention consisting of mammalian and vertebrate cDNA, although it was a species falling within the scope of those claims. *Eli Lilly*, 119 F.3d at 1567–68, 43 USPQ2d at 1405. In *Eli Lilly*, the specification and generic claims to all cDNAs encoding for vertebrate or mammalian insulin did not describe the claimed genus because they did not set forth any common features possessed by members of the genus that distinguished them from others. *Id.* at 1568, 43 USPQ2d at 1405. Nor did the specification describe a sufficient number of species within the very broad genus to indicate that the inventors had made a generic invention, *i.e.*, that they had possession of the breadth of the genus, as opposed to merely one or two such species. *Id.* The PTO has included a hypothetical example based on the facts of *Eli Lilly* in its Synopsis of Application of Written Description Guidelines in which the description requirement is not met. *See Application of Guidelines*, Example 17, at 61–64. The PTO has also provided a contrasting example of genus claims to nucleic acids based on their hybridization properties, and has determined that such claims may be adequately described if they hybridize under highly stringent conditions to known sequences because such conditions dictate that all species within the genus will be structurally similar. *See id.*, Example 9, at 35–37. Whether the disclosure provided by the three deposits in this case, coupled with the skill of the art, describes the genera of claims 1–3 and 5 is a fact question the district court did not address. On remand, the district court should deter-

2. We do not address the issue whether the breadth of the claim may implicate other validity issues, such as enablement. Only written description is before us.

mine, consistently with the precedent of this court and the PTO's Guidelines, whether one skilled in the art would consider the subject matter of claims 1–3 and 5 to be adequately described, recognizing the significance of the deposits and the scope of the claims.

[11] Enzo argues that all of the claims are adequately described on another basis, *viz.*, by means of the disclosed correlation of the function of hybridization with the bacterial DNA. In its petition for rehearing, Enzo states as attorney argument that "[t]he description and claiming of biological materials by their affinity to other materials that are clearly identified in the specification and claims (the particular deposited strains of *N. gonorrhoeae* and *N. meningitidis*) inherently specifies structure, and is routine in this field." Claim 1 sets forth the deposit numbers of six strains of *N. gonorrhoeae* to which the claimed nucleotide sequences preferentially hybridize, as well as the deposit numbers of six strains of *N. meningitidis* that are thereby distinguished. Again, as with the claimed nucleotide sequences, the sequences of the genomic DNA of those bacteria are not disclosed, perhaps because such sequencing would have been unduly burdensome at the time of Enzo's invention. '659 patent, col. 3, ll. 40–46 (noting that it would take 3,000 scientists one month to sequence the genome of one strain of *N. gonorrhoeae* and one strain of *N. meningitidis*). However, as those bacteria were deposited, their bacterial genome is accessible and, under our holding today, they are adequately described in the specification by their accession numbers. Because the claimed nucleotide sequences preferentially bind to the genomic DNA of the deposited strains of *N. gonorrhoeae* and have a complementary structural relationship with that DNA, those sequences, under the PTO Guidelines, may also be adequately described. Although the patent specification lacks description of the location along the bacterial DNA to which the claimed sequences bind, Enzo has at least raised a genuine issue of material fact as to whether a reasonable fact-finder could conclude that the claimed sequences are described by their ability to hybridize to structures that, while not explicitly sequenced, are accessible to the public. Such hybridization to disclosed organisms may meet the PTO's Guidelines stating that functional claiming is permissible when the claimed material hybridizes to a disclosed substrate. That is a fact question. We therefore conclude that the district court erred in granting summary judgment that the claims are invalid for failure to meet the written description requirement. On remand, the court should consider whether one of skill in the art would find the generically claimed sequences described on the basis of Enzo's disclosure of the hybridization function and an accessible structure, consistent with the PTO Guidelines. If so, the written description requirement would be met.

[12–14] We next address Enzo's additional argument that the written description requirement for the generic claims is necessarily met as a matter of law because the claim language appears *in ipsis verbis* in the specification. We do not agree. Even if a claim is supported by the specification, the language of the specification, to the extent possible, must describe the claimed invention so that one skilled in the art can recognize what is claimed. The appearance of mere indistinct words in a specification or a claim, even an original claim, does not necessarily satisfy that requirement. One may consider examples

from the chemical arts. A description of an anti-inflammatory steroid, *i.e.*, a steroid (a generic structural term) described even in terms of its function of lessening inflammation of tissues fails to distinguish any steroid from others having the same activity or function. Similarly, the expression an antibiotic penicillin fails to distinguish a particular penicillin molecule from others possessing the same activity. A description of what a material does, rather than of what it is, usually does not suffice. *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. The disclosure must allow one skilled in the art to visualize or recognize the identity of the subject matter purportedly described. *Id.*

[15, 16] In *Eli Lilly*, we were faced with a set of facts in which the words of the claim alone did not convey an adequate description of the invention. *Id.* at 1567, 119 F.3d 1559, 43 USPQ2d at 1405. In such a situation, regardless whether the claim appears in the original specification and is thus supported by the specification as of the filing date, § 112, ¶ 1 is not necessarily met. *See Guidelines* at 1100 (noting *Eli Lilly's* clarification of the "original claim" doctrine in situations in which the name of the claimed material does not convey sufficient identifying information). If a purported description of an invention does not meet the requirements of the statute, the fact that it appears as an original claim or in the specification does not save it. A claim does not become more descriptive by its repetition, or its longevity.

[17] Inasmuch as 112, 1 requires such description, we are not persuaded by Enzo's argument that, because the specification indicated that Enzo possessed the claimed invention by reducing three sequences within the scope of the claims t practice, Enzo necessarily described th invention. It is true that in *Vas–Cath*, w stated: "The purpose of the 'written de scription' requirement is broader than t merely explain how to 'make and use'; th applicant must also convey with reasonabl clarity to those skilled in the art that, as o the filing date sought, he or she was i possession of the invention." *Vas–Cath* 935 F.2d at 1563–64, 19 USPQ2d at 1117 That portion of the opinion in *Vas–Cath* however, merely states a *purpose* of th written description requirement, *viz.*, t ensure that the applicant had possession o the invention as of the desired filing date It does not state that possession alone i always sufficient to meet that requirement Furthermore, in *Lockwood v. American Airlines, Inc.*, we rejected Lockwood's ar gument that "all that is necessary to satis fy the description requirement is to show that one is 'in possession' of the invention.' 107 F.3d 1565, 1572, 41 USPQ2d 1961 1966 (Fed.Cir.1997). Rather, we clarifie that the written description requirement i satisfied by the patentee's disclosure o "such descriptive means as words, struc tures, figures, diagrams, formulas, etc. that fully set forth the claimed invention.' *Id.*

[18–20] The articulation of the writte description requirement in terms of "pos session" is especially meaningful when a patentee is claiming entitlement to an ear lier filing date under 35 U.S.C. §§ 119 or 120, in interferences in which the issue is whether a count is supported by the specification of one or more of the parties, and in *ex parte* applications in which a claim at issue was filed subsequent to the application. *See Vas–Cath*, 935 F.2d at 1560, 19 USPQ2d at 1114 (describing situations in which the written description requirement

may arise); *Ralston Purina Co. v. Far-Mar-Co, Inc.*, 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed.Cir.1985) (noting, in the context of claiming entitlement to the priority date of an earlier application, that the written description requirement is met if "the disclosure of the application relied upon reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter"). Application of the written description requirement, however, is not subsumed by the "possession" inquiry. A showing of "possession" is ancillary to the *statutory* mandate that "[t]he specification shall contain a written description of the invention," and that requirement is not met if, despite a showing of possession, the specification does not adequately describe the claimed invention. After all, as indicated above, one can show possession of an invention by means of an affidavit or declaration during prosecution, as one does in an interference or when one files an affidavit under 37 C.F.R. § 1.131 to antedate a reference. However, such a showing of possession alone does not cure the lack of a written description in the specification, as required by statute.

[21, 22] Similarly, we conclude that proof of a reduction to practice, absent an adequate description in the specification of what is reduced to practice, does not serve to describe or identify the invention for purposes of § 112, ¶ 1. As with "possession," proof of a reduction to practice may show priority of invention or allow one to antedate a reference, but it does not by itself provide a written description *in the patent specification.* We are thus not persuaded by Enzo's argument, relying on the PTO's Guidelines, that its disclosure of an actual reduction to practice is an important "safe haven" by which it has demonstrated compliance with the description requirement. The Guidelines state:

> Actual reduction to practice may be crucial in the relatively rare instances where the level of knowledge and level of skill are such that those of skill in the art cannot describe a composition structurally, or specify a process of making a composition by naming components and combining steps, in such a way as to distinguish the composition with particularity from all others.

Guidelines, 66 Fed. Reg. at 1101. For biological inventions, for which providing a description in written form is not practicable, one may nevertheless comply with the written description requirement by publicly depositing the biological material, as we have held today. That compliance is grounded on the fact of the deposit and the accession number in the specification, not because a reduction to practice has occurred. Such description is the *quid pro quo* of the patent system; the public must receive meaningful disclosure in exchange for being excluded from practicing the invention for a limited period of time.

CONCLUSION

For the foregoing reasons, we conclude that the district court erred in granting summary judgment that the claims of the 659 patent are invalid for failure to meet the written description requirement of 112, 1. While the district judge clearly understood and correctly applied this courts existing precedent, we nevertheless reverse because this case has taken us into new territory and we have held, as a matter of first impression, that reference in a patent specification to a deposit of genetic material may suffice to describe that material. We therefore remand for further resolution consistent with this opinion.

REVERSED and REMANDED

Stephen J. KASARSKY, Petitioner,

v.

MERIT SYSTEMS PROTECTION BOARD, Respondent.

No. 02-3006.

United States Court of Appeals, Federal Circuit.

July 17, 2002.

Postal service employee petitioned for enforcement of settlement agreement, seeking agreed-upon amount of attorney fees plus interest. The Merit Systems Protection Board (MSPB) refused to enforce agreement and dismissed petition. Employee appealed. The Court of Appeals, Linn, Circuit Judge, held that: (1) as a matter of first impression, petition for enforcement of settlement agreement must be filed within reasonable time after petitioner becomes aware of alleged breach of agreement; (2) Postal Service breached settlement agreement when it failed to pay attorney fees as promised; and (3) breach occurred when Postal Service failed to meet deadline set by employee.

Reversed and remanded.

1. Officers and Public Employees ⇐72.36

A petition for enforcement by Merit Systems Protection Board (MSPB) alleging breach of a settlement agreement must be filed within a reasonable amount of time from the date on which the petitioning party becomes aware of a breach of the agreement, and the reasonableness of the time period depends on the circumstances of each case.

2. Officers and Public Employees ⇐72.36

A petition for enforcement of a settlement agreement by Merit Systems Protection Board (MSPB) is presumed to be timely and subject to the MSPB's jurisdiction until challenged, either by MSPB on its own initiative or by the agency.

3. Officers and Public Employees ⇐72.36

In a challenge to the timeliness of petition for enforcement of settlement agreement by Merit Systems Protection Board (MSPB), if on its face the petition is untimely, the burden is on petitioner to show facts establishing why the petition is not untimely.

4. Compromise and Settlement ⇐2

Disputes involving settlement agreements are governed by contract principles.

5. Contracts ⇐315

A "breach of contract" is simply the non-performance of a contractual duty. Restatement (Second) of Contracts § 235(2).

> See publication Words and Phrases for other judicial constructions and definitions.

6. Contracts ⇐277(1), 313(1)

When no time for performance is specified and one party performs, the non-performing party is not in breach of the contract until either (1) the performing party demands performance within a reasonable amount of time, and the other party still fails to perform within the time specified; or (2) the non-performing party repudiates the contract, and the performing party chooses to treat the repudiation as a breach.